U-PLEX®

Human OX40/TNFRSF4

Product Options	Catalog Number	Description
Multiplex	K151AEM, K251AEM	U-PLEX Immuno-Oncology Group 1 (hu)
Singleplex	K151T7K-1/-2/-4	U-PLEX Human OX40/TNFRSF4 Assay with SECTOR™ plates
	K151T7K-21/-22/-24	U-PLEX Human OX40/TNFRSF4 Assay with QuickPlex® plates
	K251T7K-2/-4	U-PLEX Human OX40/TNFRSF4 Assay with 384-well plates
Antibody Set	B22T7-2/-3	U-PLEX Human OX40/TNFRSF4 Antibody Set
Protocol	U-PLEX Product Inserts are available at www.mesoscale.com	

The MESO SCALE DISCOVERY® U-PLEX platform was designed to provide ultimate flexibility for detection of biomarkers in a wide variety of sample types. This datasheet provides the representative performance of the U-PLEX® Human OX40/TNFRSF4 Assay tested on U-PLEX 96-well plates run as a multiplex. The data do not represent the product specifications. Under your experimental conditions, the assay may perform differently from the representative data. U-PLEX assays are offered in either singleplex or multiplex; both are available on 96- or 384-well plates. See a U-PLEX product insert for instrument compatibility.

Representative Calibration Curve and Sensitivity

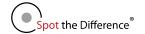| Assay	Median LLOD (pg/mL)	LLOD Range (pg/mL)
OX40/TNFRSF4	0.26	0.12-0.38

The Calibrator curve was fitted with a 4-parameter logistic model with a $1/Y^2$ weighting. The lower limit of detection (LLOD) is a calculated concentration corresponding to 2.5 standard deviations above the background (zero Calibrator).

Precision

Control	Average Conc. (pg/mL)	Average Intra-run Conc. (%CV)	Inter-run Conc. (%CV)
High	244	3.9	5.3
Mid	56	3.9	7.7
Low	13	5.2	12.4

Controls were made by spiking Calibrator into assay diluent at 3 levels within the quantitative range of the assay. Average intra-run concentration %CV is the average %CV of the control replicates within an individual run. Inter-run concentration %CV is the variability of controls across multiple runs.

Spot the Difference®

MSD® U-PLEX Human OX40/TNFRSF4

Tested Samples

Sample Type	Serum (N=10)	EDTA Plasma (N=10)	Normal Lysate (N=5)	Tumor Lysate (N=5)
Median (pg/mL)	101	99	32	406
Range (pg/mL)	49-155	74-134	15-154	27-1,310
% Detected	100	100	100	100

Normal serum and plasma samples were diluted 4-fold prior to the assay. Lysates were tested at a protein concentration of 0.5 mg/mL.

Dilution Linearity

Serum			EDTA Plasma		
Fold Dilution	Average % Recovery	% Recovery Range	Fold Dilution	Average % Recovery	% Recovery Range
2	109	104 - 114	2	107	87 - 115
8	95	91 - 98	8	98	91 - 112
16	90	89 - 95	16	99	87 - 121

Normal human serum and EDTA plasma were spiked with Calibrator and tested at different dilutions. Percent recovery at each dilution level was normalized to the dilution-adjusted, 4-fold concentration. Samples may benefit from additional dilution with assay diluent to reduce matrix effects.

% Recovery = (measured concentration / expected concentration) x 100

Spike Recovery

Spike Level	Serum		EDTA Plasma	
	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
High	100	93 - 105	95	67 - 107
Mid	92	67 - 105	98	71 - 107
Low	113	96 - 148	97	74 - 105

Normal serum and plasma were spiked with Calibrator at 3 levels. Spiked samples were diluted 4-fold to determine the expected concentration of the analyte. Samples may benefit from additional dilution with assay diluent to reduce matrix effects.

% Recovery = (measured concentration / expected concentration) x 100

Specificity

To assess specificity, the OX40/TNFRSF4 Antibody Set was tested individually against a larger panel of analytes for nonspecific binding (APRIL/TNFSF13, BAFF-R/TNFRSF13C, BCMA/TNFRSF17, BDNF, C-Peptide, CD20, CD27, CD28, CD40L (soluble), CD276/B7-H3, CTACK, CTLA-4, Desghrelin, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, EPO, E-Selectin, FGF (basic), FGF-23, FLT3L, Fractalkine, FSH, Galectin-9, G-CSF, GITRL/TNFSF18, GITR/TNFSF18, Ghrelin (Ser3-octanoylated), gp130 (soluble), GIP (1–42), GIP (3–42), GLP-1 (7–36), GLP-1 (9–36), GM-CSF, Granzyme A, Granzyme B, GRO-α, HAVCR2/TIM-3, HVEM/TNFRSF14, ICOS, ICOS-L/B7-H2, I-309, IFN-α2a, IFN-β, IFN-γ, IL-1α, IL-1β, IL-1RA, IL-2, IL-2Rα, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17A/F, IL-17C, IL-17D, IL-17E/IL-25, IL-17F, IL-18, IL-21, IL-22, IL-23, IL-27, IL-29/IFN-λ1, IL-31, IL-33, Insulin, IP-10, LAG3, Leptin, LH, LIGHT/TNFSF14, MCP-1, MCP-2, MCP-4, M-CSF, MDC, MIF, MIG, MIP-1α, MIP-1β, MIP-5, MMP-1, MMP-2, MMP-7, Nectin-4, OX40/TNFRSF4, PD1, PD-L1, PD-L2, Pentraxin 3, Perforin, PIGF, PP, Proinsulin, proMMP-9, P-Selectin, PYY (3–36), RAGE (soluble), RANKL/TNFSF11, RANTES, S100A12, SDF-1α, Tie-2, TIGIT, TLR1, TNF-α, TNF-β, TNF-RI, TNF-RII, TPO, TRAIL, TSLP, VEGF-A, VEGF-D, VEGFR-1/Flt-1, and YKL-40). Nonspecific binding was less than 2.0%.

% Nonspecificity = (nonspecific signal / specific signal) x 100

Diluent Compatibility

Diluents 58 and 3 are provided with this assay. MSD offers a range of assay and antibody diluents for separate purchase. Depending on your assay needs, other diluents may be tested.

Assay Components

Calibrator: OX40/TNFRSF4 is included in Calibrator 20 The human OX40/TNFRSF4 Calibrator is OX40 (29–216) expressed in a human cell line.
Antibodies: The U-PLEX Human OX40/TNFRSF4 Assay uses a mouse monoclonal antibody for capture and a goat polyclonal antibody for detection.
Assay generation: A

Note: This datasheet contains representative assay performance data. In custom multiplex formats, the assay may perform differently from the representative data shown.